Reduction of Copper(II) Complexes by Electron Capture in an Electrospray Ionization Source

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The relative proportion of 1:1 Cu(I)– and Cu(II)–peptide complexes $PeptCu(I)^+$ and $[Pept - H+Cu(II)]^+$ yielded by electrospray ionization of copper sulfate and GlyHisLys solutions in water/methanol was examined under different source conditions. Two factors leading to an increase in Cu(I) complex ratio were found. (1) Increase of nozzle–skimmer voltages caused collision-induced dissociation of Cu(II) complexes, and most probably favor ligand-to-metal electron transfers that result in the decoordination of oxydated ligands to form $PeptCu^+$. (2) Independent of these "innersphere" processes that involve only electron exchange inside the coordination sphere around the metal cation, an increase in source voltages with a concomitant increase of current and, supposedly, electron counterflow between the counterelectrode and the capillary caused an increase in $PeptCu^+$ relative proportion. The hypothesis that an "outersphere" electron capture might happen in these conditions was verified by using discharge supressing SF_6 gas as nebulizing gas. The electronegative gas reduced the current brought on by high voltages and inhibited the $PeptCu^+$ increase phenomenon. (J Am Soc Mass Spectrom 1998, 9, 1217–1221) © 1998 American Society for Mass Spectrometry

Electrospray ionization mass spectrometry (ESI-MS) is a rapidly expanding technique, and there is no doubt as to its huge potential for analytical work and for fundamental studies. The mechanisms of ion formation from charged droplets has been the object of numerous studies [1–4] among which is one by Blades et al. [2] who focused on the electrospray source as an electrolysis cell. Their generally accepted interpretation of the processes occurring in the electrospray source states that the imposed electric field leads to a partial separation of positive from negative electrolyte ions present in solution. In the positive mode, the spray droplets carry off an excess of positive ions. The transfer of electrons to complete the electric circuit implies (1) that an electrochemical oxidation occur at the solution–metal interface of the capillary, whereas (2) a concomitant reduction should happen on the counterelectrode. Of these two processes, mostly the electrochemical oxidation reaction occurring at the solution–metal interface of the capillary tip has been studied and exploited. The first evidence of an oxidation reaction was provided by inserting a zinc capillary in the probe, which resulted in the production of Zn^{2+} ions solvated by dimethylsulfoxide (DMSO) [2]. Later, Van Berkel et al. [5–8] used the same concept of an electrochemical oxidation to explain the presence of radical cations, notably of polycyclic aromatic hydrocarbons (PAH) and metalloporphyrins. Cole et al. [9–11] extended this observation to several electrochemical reaction studies. To our knowledge, however, the complementary part of the electrolysis cell, the reduction that should happen on the counterelectrode, has not been studied.

In the course of our studies on the gas-phase interactions of metal cations and peptides [12], we found a gas-phase reduction reaction of Cu(II) complexes to Cu(I) species. This has been observed earlier by others [13–17] and could be partially attributed to charge transfer, that is to say, "innersphere" ligand to metal electron transfer reactions [12]. We wish to report here a second possible reduction mechanism (which might be called "outersphere") that happens in the electrospray source, when a high electric field is applied between the capillary and the counterelectrode. In addition to nozzle–skimmer effects, we, therefore, investigated the influence of source voltages on the relative proportion of Cu(II) and Cu(I) complexes of a copper-

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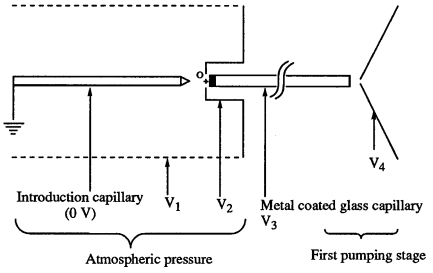


Figure 1. Schema of the electrospray source.

binding peptide GlyHisLys. Discharge supressing gas SF_6 was used to probe the effect of the counterflow electrons accompanying the high voltage, under corona-dischargelike conditions.

Experimental

The tripeptide GlyHisLys (GHK, acetate salt) was purchased from Sigma (St Quentin-Fallavier, France), and the copper sulfate pentahydrate from Aldrich (St Quentin-Fallavier, France). Solutions of Cu^{2+} and peptide mixtures were prepared with water/methanol 1/1 v/v as solvent (concentration around 4×10^{-5} mol/L). SF_6 gas was purchased from Messer France S. A. (Nitry-Mory, France).

Solutions were infused through a syringe pump at a flow rate of 50 μL/min to an electrospray source (Analytica of Branford, Branford), coupled to a Nermag L 1010 quadrupole mass analyzer. A scheme of the source is depicted in Figure 1. The three source voltages V_1 (cylinder), V_2 (counterplate), and V_3 (metal coated transfer capillary) were varied together. Alternatively, dinitrogen and sulfur hexafluoride were used as nebulizing gases, at a pressure of approximately 2.7×10^5 Pa. As usual for electrospray sources, the voltage V_4, applied on the extracting cone at the end of the transfer capillary, may be varied to achieve some fragmentations [so called nozzle–skimmer collision-induced decompositions (CID)]. These are induced by collisions with the gas molecules present in this medium vacuum area, before the entrance to the single quadrupole analyzer. Our spectral data were averaged over a 60-s acquisition.

The MS/MS experiments were recorded on a Quattro II (Micromass, Manchester, UK) mass spectrometer, using argon as collision gas, and nitrogen as nebulizing and drying gas. All the effects described below were also observed on this apparatus.

Results

Nozzle–skimmer CD

Consistent with previous studies [13–17], the direct ESI-MS mass spectrum, of peptide (abbreviated to "Pept") and copper sulfate solutions yielded a mixture of Cu(II) complexes, such as $[Pept - H+Cu(II)]^+$ (m/z 402 and

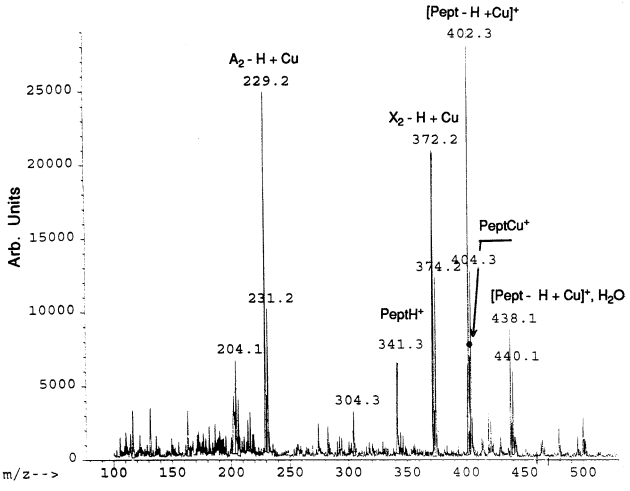


Figure 2. Overview of the direct ES mass spectrum in standard conditions. ($V_1 = -2500$ V, $V_2 = -3300$ V, $V_3 = -4000$ V, $V_4 = 130$ V, nebulizing gas N_2). M here stands for the tripeptide.

m/z 404), and Cu(I) adducts like $PeptCu(I)^+$ (m/z 403 and 405) (Figure 2). Similarly to what has been observed on histidine/metal complexes [12], these two 1:1 peptide/metal complexes should arise from the fragmentation of higher mass precursor ions, as there are no precursor ion/fragment ion relationship between the two.

The relative proportion of Cu(II)– and Cu(I)–GHK 1:1 complexes was found to be altered by variations of nozzle–skimmer voltages V_4 (Figure 3). High cone voltages resulted in a diminution of $[Pept - H+^{63}Cu]^+$ abundance, whereas that of $Pept^{63}Cu^+$ increased. Concomitant to this phenomenon, a global increase of the intensity of ions of smaller mass-to-charge ratio was observed. Typically, an increase in cone voltage V_4 induces reactive collisions in the high gas density region (~10 Pa) existing between the transfer capillary and the extracting cone. All changes in the mass spectra might therefore be related to gas-phase reactions happening in that zone.

To find a way to give tangible evidence of these gas-phase collisions, some MS/MS experiments were

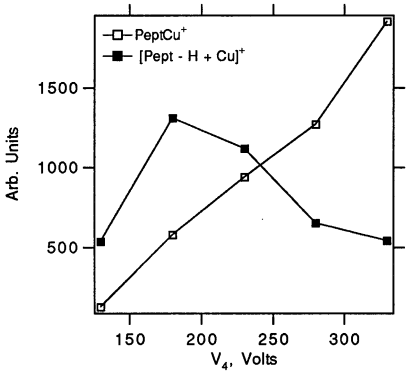
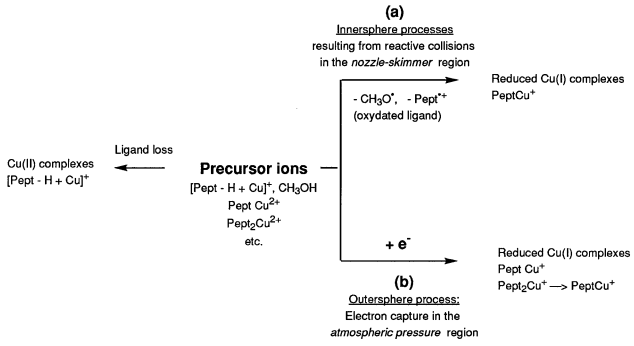


Figure 3. Variation of $PeptCu^+$ and $[Pept - H+Cu]^+$ abundances with the nozzle–skimmer potential V_4 ($V_1 = -2500$ V, $V_2 = -3200$ V, $V_3 = -4000$ V).

Table 1. Reactions brought on by adjustment of the nozzle–skimmer potential. Relative proportions of 1:1 peptide–copper complexes and fragment/parent ratios, in the mass spectra recorded at low source voltages ($V_1 = -2500$ V, $V_2 = -3300$ V, $V_3 = -4000$ V)

Nozzle–skimmer voltage (V_4)	$PeptCu^+$ / $[Pept - H+Cu]^+$	a_2^* / $PeptCu^+$	x_2^* / $[Pept - H+Cu]^+$
130V	0.2	3.6	0.7
180V	0.4	3.6	2.0
230V	0.8	1.8	1.3
280V	1.9	1.1	2.0
330V	3.6	1	2.1



Scheme 1

carried out. CID experiments on the ions $[Pept - H+{}^{63}Cu]^+$ and $Pept^{63}Cu^+$ showed that the two complexes have very different fragmentation patterns: the Cu(II) complex yielded m/z 372 as unique fragment ion, whereas the CID spectra of the $Pept^{63}Cu^+$ ion showed one unique peak at m/z 229, and the collision energy used to obtain fragmentation was slightly higher (20 eV for $PeptCu^+$ versus 14 eV for $[Pept - H+Cu]^+$ in the laboratory frame of reference). Experiments in deuterated solvents led to a shift of 3 and 5 u for m/z 229 and 372, respectively. The tripeptide GHK, having a total of eight exchangeable hydrogens, in its neutral form, we assumed that m/z 372 should correspond to the loss of a $H_2N–CH_2^{\cdot}$ radical from the N-terminal glycine of $[Pept - H+Cu]^+$, and named the ion $[x_2 - H+Cu]$ or x_2^*. The m/z 229 ion, assigned as the loss of the C-terminal lysine and CO in an a-type fragmentation, will be refered to as $[a_2 - H+Cu]$ or a_2^* (Roepstorff and Folhman nomenclature [18]).

Beside the $PeptCu^+/[Pept - H+Cu]^+$ proportion, Table 1 lists the values calculated for the $x_2^*/[Pept - H+Cu]^+$ and $a_2^*/PeptCu^+$ ratios in the source mass spectra at various cone voltages. Calculations of these latter two ratios was meant to roughly probe the rate of reactive collisions inducing gas-phase fragmentations. It worked well with the $x_2^*/[Pept - H+Cu]^+$ ratio which increased as expected (with the exception of one value); this was indeed consistent with an increase in the number of reactive collisions which caused a decrease in precursor ion $[Pept - H+Cu]^+$ and the formation of more x_2^* fragment ions. The $a_2^*/PeptCu^+$ ratio, on the other hand, was found not to reflect the collision rate induced by the increase of nozzle–skimmer voltage in the same straightforward way. In fact, it mainly decreased as a result of the increase in $PeptCu^+$ abundance. This showed that the precursor ion, here, was formed faster than it fragmented, which meant its gas phase chemistry should be completely different from that of $[Pept - H+Cu]^+$. Nevertheless, the variations of these ratios reflected the fact that important changes occur in the mass spectra upon variation of the nozzle skimmer voltage V_4, and, therefore, did give evidence that some gas phase reactions in the vicinity of the extracting cone occurred. Reactions in the nozzle–skimmer region could, therefore, also be largely responsible for the change in abundance ratio of the Cu(I)/Cu(II)–peptide 1:1 complexes.

Gas-phase collisions in the nozzle–skimmer region may cause a variation of the abundance ratio of Cu(I)/Cu(II)–peptide 1:1 complexes in a variety of different ways. First, the $[Pept - H+Cu]^+$ ion might require lower energies and fewer collisions to fragment, its relative intensity would decrease more rapidly than that of the $PeptCu^+$ ion. This was corroborated by the collision energies used for our CID experiments: the $[Pept - H+Cu]^+$ required 6 eV less to fragment (energy in the laboratory frame of reference). Second, the $PeptCu^+$ adducts might result from the fragmentation of other copper complexes, which require high energy to fragment. Precursor ion scans of $PeptCu^+$ ions were recorded, but no significant precursor ion could be detected. In a previous study [12], however, precursor ion scans on adducts of L-histidine showed the reduced $HisCu^+$ complex resulted, at high cone voltage, from the fragmentation of several complexes, either partially solvated by methanol $[(His - H)^- Cu^{2+} (CH_3OH)]$ or doubly charged (His_2Cu^{2+}). In all cases, consistent with what has been reported by others [13–17] and us [12], the presence of reduced $PeptCu(I)^+$ complexes in these conditions may be largely attributed to collision-induced processes in the medium vacuum area of the source that cause ligand-to-metal "innersphere" electron transfers and decoordination of odd-electron species ($CH_3O^{\cdot}$, $Pept^{+\cdot}$) (Scheme 1a).

Field Effects

Independent of these nozzle–skimmer effects, it was found that different source voltages also yielded different abundance ratios for Cu(I)/Cu(II) complexes. This is shown in Figure 5a which depicts the $PeptCu^+$ and $[Pept - H+Cu]^+$ abundances at various source conditions. The voltages V_1, V_2, and V_3 were varied together and the extracting cone voltage V_4 was maintained at 230 V. With this medium value on the extracting cone, both Cu(I) and Cu(II) complexes were detected in significant quantities because, as described in the preceding paragraph, some reduction processes occur in the nozzle–skimmer region. Some SIMION [19] calculations were done to follow the effect of each increase in

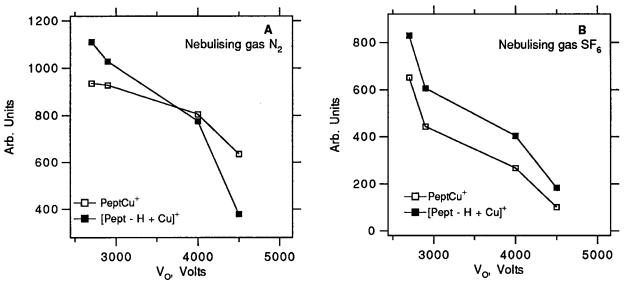


Figure 4. Variation of $PeptCu^+$ and $[Pept - H+Cu]^+$ abundances with the electric field in the source. (**A**) Nitrogen was used as nebulizing gas, (**B**) hexafluoride sulfur was used as nebulizing gas. For simplification purposes, the field in the source is represented by the potential (V_O) at the point O, defined in Figures 1 and 4.

voltage V_1, V_2, and V_3. For a simplification purpose, the O point potential (Figure 1) was chosen as reference for Figure 4 and Table 2. Figure 4a shows that an increase in source potentials resulted in a global diminution of absolute abundances. This was expected as a change in source voltages altered ion optics and diverted some ions away from the transfer capillary. Diminution of $PeptCu^+$ complex abundance, however, was much slower than that of $[Pept - H+Cu]^+$. To see if some ion/molecule collisions might be involved, the ratios $x_2^*/[Pept - H+Cu]^+$ and $a_2/PeptCu^+$ were calculated. The variations of these ratios (Table 2) were different from the ones previously observed in Table 1: the $x_2^*/[Pept - H+Cu]^+$ ratio virtually did not change, or tended to diminish, showing that no reactive collisions resulting in the fragmentation of $[Pept - H+Cu]^+$ occured. The $a_2^*/PeptCu^+$ decreased essentially as a result from the increase in the abundance of $PeptCu^+$ ion but, globally, little change was observed in the mass spectra in the fragment ions region. It was, therefore, concluded that the increase in the abundance of Cu(I)/peptide complex could not be explained, here, solely by ion–molecule collisions in the source.

Under conditions of high source voltages, an increase in current intensity was also observed: a current of 25 μA was measured at the highest voltages used with dinitrogen as nebulizing gas, but no detectable arcing was observed. Gaseous electronics theory [20, 21] defines corona discharge as an "avalanche" which must be initiated by an electron, either emitted from the cathode or detached from a negative ion. It is characterized by an abrupt increase in the current between the electrodes (from about 10^{-14} to 10^{-6} A). The increase in current, observed in our experiment may be, therefore, largely due to a counterflow of electrons in direction of the capillary. These electrons could be emitted by the cathode, as a secondary emission to the impact of the droplets diverted away from the glass capillary entrance, or they could be detached from the negative ions present in small proportion in the droplets. In all case, the positive and negative entities should diffuse through the gas under the influence of the applied electric field and possibly recombine with each other. The energy of these entities is unknown, although one could imagine that it should partially depend on the length and time during which they "fly," accelerated by the electric field. These values could not be very high under atmospheric conditions.

Our hypothesis was therefore, that an electron (or a negative ion) might induce a reduction of Cu(II) species by direct electron capture, in the atmospheric pressure region of the source, before the entrance in the glass capillary. A reduction by electron capture could happen from a variety of precursor ions (Scheme lb).

To verify this hypothesis, discharge supressing, electronegative, SF_6 gas was used instead of dinitrogen. In electronegative gases, the initiation of the electron avalanche is impaired, the current carriers are essentially negative ions whose diffusion is slower than that of electrons [20]. Also, if the electron capture happens through charge exchange from a negative ion, it is less likely to happen with $SF_6^{-\cdot}$ as hexafluoride sulfur has a relatively high electron affinity (around 1.1 eV [22]). The results, illustrated in Figure 5b, show that with SF_6 gas in the source the Cu(I)/Cu(II) complexes ratio stayed virtually the same. Hexafluoride sulfur, acting as insulator, limited the current to undetectable values. All observations were therefore consistent with the hypothesis that, in the absence of free electrons or favorable negative ions, no reduction by electron capture in the atmospheric region of the source could happen (Scheme 1b). Only the "innersphere" reduction processes (Scheme 1a), described in the first paragraph, are responsible for the Cu(I) complexes observed.

Dinitrogen and hexafluoride sulfur have about the same ionization potential (15.6, and 15.3 eV, respectively [22]), so a direct charge exchange with the nebulizing gas cannot account for the differences in variation of the Cu(I)/Cu(II) abundance with both gases. One could argue that a complex may be formed that could facilitate the charge transfer. It is true that dinitrogen fixation by copper complexes have been reported [23] but these are said to be formed, in the nozzle–skimmer region, by reaction on a previously reduced $[Cu(I)diimine]^+$ ion that results from CAD of a Cu(II) complex. No adduct either with dinitrogen or hexafluoride sulfur were detected in our experiments. Furthermore, the fragment/parent ratios, $x_2^*/[Pept - H+Cu]^+$ and $a_2/PeptCu^+$, calculated with SF_6 vary only very slightly, thus confirming that no significant increase in CID occurred at higher source voltages (Table 2).

Conclusion

To our knowledge, this is the first evidence of a direct electron capture in an electrospray source. Although electron capture has been much encountered before in mass spectrometry, in the negative mode or by electron exchange with target inert gases, its occurence in these conditions, that might be described as intermediate between ESI and atmospheric pressure chemical

Table 2. Relative proportions of Cu(I)/Cu(II) complexes and fragment/parent ratios for nitrogen and hexafluoridesulfur nebulizing gases upon variation of source voltages (V_1, V_2, V_3). For simplification purposes the field is represented by the potential at the O-point defined in Figure 1 (V_O)

	Nebulizing gas					
	N_2			SF_6		
	$PeptCu^+$	a_2^*	x_2^*	$PeptCu^+$	a_2^*	x_2^*
Ratio	$[Pept - H+Cu]^+$	$PeptCu^+$	$[Pept - H+Cu]^+$	$[Pept - H+Cu]^+$	$PeptCu^+$	$[Pept - H+Cu]^+$
$-V_O$ (V)						
2700[a]	0.8	3.6	1.3	0.8	1.7	1.1
2900[b]	0.9	1.4	1.3	0.7	1.8	1.1
4000[c]	1.1	1.1	1.2	0.7	2.0	1.0
4500[d]	1.7	0.7	1.1	0.5	2.2	1.1

[a] $V_1 = -2500$ V, $V_2 = -3300$ V, $V_3 = -4000$ V.
[b] $V_1 = -2500$ V, $V_2 = -3400$ V, $V_3 = -4500$ V.
[c] $V_1 = -2500$ V, $V_2 = -4900$ V, $V_3 = -5500$ V.
[d] $V_1 = -3200$, $V_2 = -5200$ V, $V_3 = -6900$ V.

ionization, is new. Although these conditions are out of range from standard operation, they may be reached quite easily, without one necessarily being aware of them, as the probe is not always visible and current measurements are not always available.

This finding was prompted by the peculiar chemistry of metals adducts, in which electron transfer redox reactions seem to be recurring processes [12–15]. In this case, the electron captured being "outersphere" (that is, outside of the sphere of coordination of the metallic center), the resulting reduction should be largely independent of the ligand. In fact, similar effects have been observed for other complexes of Cu(II) with amino acids and peptides. Investigations on other metals, notably Fe(III)/Fe(II), should help to characterize the phenomenon.

Unexplained reduction of metal(II) salts to metal(II) species have been noted in desorption ionization sources [24–28]. It had suggested this might arise from electrons present in the plasma created by the impact of the primary particle [29]. The evidence of an electron capture happening in electrospray source brings an additional argument in favor of this hypothesis.

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